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DEPARTMENT OF MICROBIOLOGY

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"THE ROLE OF THE MACROPHAGE IN THE IMMUNE RESPONSE: THE EFFECT OF TOBACCO PRODUCTS ON MACROPHAGE FUNCTION."

10035457

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Abstract

Male Balb/c mice were exposed to various protocols involving acute exposure to Kentucky Reference cigarettes. The animals were exposed to smoke both before and after administration of antigen. The type, number and duration of exposures to smoke were varied. Three parameters of immune responsiveness were studied: (1) the number of direct and indirect plaque-forming cells (PFC), (2) the primary and secondary serum antibody response, and (3) the response to mitogenic stimulation.

The following findings were obtained:

1. Exposure of mice to Kentucky Reference 1A1 cigarettes in each of the various protocols tested was without effect on immune responsiveness.

2. Sham-smoked animals had a reduced immune responsiveness compared to untreated bench controls.

3. Exposure of mice to Kentucky Reference 1R1 cigarettes caused significant suppression of the appearance of both direct and indirect PFC.

4. Exposure to 2 or 3 of the 1R1 cigarettes caused greater suppression than a single cigarette.

5. Animals removed from the smoking protocol after antigen administration had a partial recovery of immune responsiveness.

6. Exposure to 1R1 cigarettes reduced the primary serum hemagglutinating antibody response, but under the conditions studied had no effect on the secondary response.

7. Exposure of mice to the 1R1 cigarettes for either 7 or 15 days had no effect on the ability of spleen cells to respond to the mitogenic stimulation of Concanavalin A or Phytohemagglutinin.

These results suggest that the smoking of high nicotine-containing cigarettes has a deleterious effect on the number of antibody-forming cells in mouse spleen populations. Further experiments are in progress to define the cells affected and to determine possible mechanisms involved.

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Progress Report No. 3

Male Balb/c mice were exposed to various [illegible]

There have been a large number of studies carried out which have suggested that components of cigarette smoke may have direct carcinogenic activity. In addition, while containing a variety of potential carcinogens, components of cigarette smoke may also exert a suppressive effect on the immune system affecting both local immunity in the lung and systemic immunity. Therefore, the smoking of cigarettes may contribute to the initiation of a neoplastic event as well as make the individual more susceptible to infection as a consequence of chronic impairment of the normal defense mechanisms operating in the respiratory tract. In this regard, the alveolar macrophage plays a dominant role in the defense of the lung against microbial infection. It also has a prominent role in the clearance of particulate matter of many different types from the lung. Although there is local antibody (IgA) produced in the lung, the major organ of antibody formation in response to intratracheal instillation of immunogen is not the lung. It has been suggested that foreign materials deposited in the lung parenchyma are readily eliminated or rendered non-immunogenic, and that only a small proportion of the immunogen enters the blood stream and triggers an immune response in the spleen. The site of passage of the immunogenic material is thought to be in the alveolar space which is occupied by macrophages. One of the functional roles of macrophages which can be included in a long list of yet ill-defined roles, is that of transport and/or presentation of antigen to antibody-forming cells. It is very possible that the ability of the alveolar macrophage to carry out its normal defense functions might be severely affected by the presence of a wide variety of environmental pollutants that enter through the lung. Previous studies have shown that chronic exposure to sulphur dioxide and, in some cases carbon, has resulted in impairment of the immune response of experimental animals to inhaled immunogens. Another environmental pollutant, cigarette smoke, or extracts thereof, has been shown to affect the morphology of macrophages along with a number of their biochemical and functional properties. In addition, young adult cigarette smokers have been found to have significantly lower concentrations of serum antibody to influenza A_2 virus after either natural infection via the respiratory tract or vaccination than found in a similar group of nonsmoking controls. These studies suggested that cigarette smoking might have a detrimental effect on the immune system.

The rationale employed in these studies was based on the argument that macrophage participation in the immune response is a function of the physical nature of the immunogen. Macrophages appear to be necessary for immune responses to multideterminant, complex immunogens. In addition, immune responses to these materials also require the cooperative effect of thymus-derived cells. Therefore, the use of a complex antigen such as SRBC or KLH, and the observation of a suppressed immune response following exposure to smoke, would enable us to study mechanisms, at the cellular level.

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The initial period of this grant was spent in establishing a system in which we could measure antibody production by single cells against KLH. Details of the hemolytic plaque assay used for the detection and enumeration of individual cells either synthesizing or releasing antibody to KLH were presented in a previous

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progress report and the published paper is appended to the grant renewal request. In the second year of the grant, we attempted to establish techniques in which heterogeneous cell populations could be separated into individual functional cell types. Our particular interest at that time was directed toward the preparation of a potent and specific anti-macrophage serum to be used to deplete cell populations of macrophages so that we could determine whether the immune response to KLH was dependent upon the presence of accessory cells. Details of our work with rabbit anti-mouse macrophage serum were given in Progress Report No. 2 and a copy of our manuscript which was recently submitted to Immunology is appended. We extended the anti-macrophage serum work to the rabbit system and have established that the in vitro anamnestic response of rabbit popliteal lymph node cells is also macrophage-dependent. A copy of an abstract presented at the 59th Annual Meeting of the American Association of Immunologists in April, 1975 is also included in the grant application. Also, during the second year of the grant we extended out studies of the fundamental concepts of antibody formation into areas peripheral to the mission of this grant but which may have some bearing on our results. In Progress Report No. 2 we provided some information on the use of an anti-plasma cell serum to study cellular differentiation of the murine plasma cell. A copy of the first of at least three publications from this work, submitted (Journal of Immunology) or to be submitted, is attached. These studies have also led us into work with T-independent antigens and served as impetus for us to begin a colony of nude (Thymus-deficient, nu/nu) mice. In a second series of studies, we were interested in studying potential regulatory mechanisms operating in the immune response. We have observed that Prostaglandin E_1, but not other prostaglandins, enhanced antibody formation in our in vitro anamnestic response system. These studies could eventually be extended to immunocompetent cells found in lung tissue. A copy of our manuscript submitted to Prostaglandins is attached to the grant application.

The experiments described in this Progress Report were initiated in late October, 1974 upon receipt of the Prototype Mark II Walton Horizontal Smoke Exposure Machine. Initial attempts were made to calibrate the machine to [illegible] settings indicated in the manual. Rotometer was set at 14 to achieve a 35 ml puff volume, purge set at 10. The machine was set to give a 2 sec. puff, 28 sec. mix and 30 sec. purge to complete the cycle. All experiments were done with Kentucky Reference cigarettes, either 1A1 or 1R1, which had been stored at -20°C prior to use. One week before use, the cigarettes were removed from the freezer, packages opened and stored in a humidified chamber at room temperature. Exposure consisted of the first 8 puffs from the cigarettes. Male Balb/c mice were used as the experimental animal for several reasons. Although they contain endogeneous type A and type C virus particles, spontaneous induction of leukemia is not characteristic of Balb/c mice as with other inbred strains. Secondly, Dr. Kreisher suggested that male Balb/c mice be used due to low inducibility of aryl hydrocarbon hydroxylase, an enzyme involved in the metabolism of aromatic hydrocarbons and thought to be related to the carcinogenesis of tobacco products. Before each experiment, the animals were acclimated to the smoking machine by putting them through 8 puff cycles, two times a day for a period of one week. Those animals that were to be used in smoking experiments were then exposed to increasing numbers of puffs of cigarette smoke for 2 days before they entered a smoking protocol. It should be noted that there were few deaths, probably due to intoxication when the 1A1 cigarettes were used. A mortality

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rate of about 15-20% was obtained using 1R1 cigarettes. As will become evident as the data is discussed, the sham-smoked animals responded in a diminished fashion when compared to the untreated, bench control animals. All other methods are as described in the grant application. Our initial experiments were concerned with obtaining an understanding of the effects of cigarette smoke on the number of spleen plaque-forming cells which is a direct measure of antibody-forming cells, on serum antibody levels in both primary and secondary immune responses and on the ability of spleen cells to respond to mitogenic stimulation. These experiments have concentrated on acute phase of smoke exposure with antigen being injected before, along with or after exposure to smoke. Some of the parameters studied were type of cigarette (1A1 vs. 1R1), the number of cigarettes used, duration of exposure to smoke and effect of withdrawal of animals from smoking protocols.

In the first series of experiments, male Balb/c mice were injected with KLH on day 0 and smoking of one 1A1 cigarette twice each day for 5 days was begun at the same time. On the 5th day, spleens were removed from each of 6 smoked, 6 sham-smoked and 4 bench control animals. Single cell suspensions were prepared and spleens were assayed individually for direct (IgM) and indirect (IgG) PFC. Table 1 shows that both smoked and sham-smoked animals had a loss of weight after being placed on the smoking machine. Again, it will be pointed out that there was a reduction in the number of PFC even in sham-smoked animals compared to bench controls. These results indicate that some of the suppression of immune responsiveness may be due to the stress conditions placed on experimental animals. The number of PFC in smoked and sham-smoked animals is not significantly different indicating that the approximate 25% suppression of responsiveness is probably due to stress conditions. Tables 2 and 3 show the results of experiments in which animals were exposed to smoke as above for 7 days and then injected with KLH. In the former experiment the animals were rested (no smoke) for the following 5 days, while, in the latter, smoke was continued according to the same protocol until the time of assay, day 12. It should be noted that in neither was there significant suppression of the immune response in smoked animals.

In the next series of experiments, animals were injected with KLH on day 0 and smoke exposure was begun 1, 2, 3 or 4 days later and continued until the time of assay, day 5. It can be seen in Tables 4-7 that there was no immunosuppressive effect of smoking a single 1A1 cigarette twice each day when direct PFC were enumerated. In some cases, Tables 5 and 7, it appears that smoking suppressed antibody formation; however, sham-smoked animals also had depressed immune responses, again suggesting that the reduction in immunocompetence was a function of stress conditions.

In view of the findings that a single 1A1 cigarette was ineffective in having an influence on the immune response of the mice we decided to increase the number of cigarettes used for each exposure. One possible reason for lack of effect of the 1A1 could have been related to dose of smoke that the animals received. Table 8 shows the effect of giving antigen 1 day before exposure to 2 cigarettes. It can be seen that increasing the smoke dose of 1A1 cigarettes

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had no effect on the immune responsiveness of test animals. Table 9 shows the results of experiments in which mice were exposed to smoke for 5 days, then injected with KLH and were rested for the time before spleens were assayed for PFC. It can be seen that increasing the smoke dose had no effect on either direct or indirect PFC. Although not shown, a similar experimental design in which the animals were exposed to smoke from 2 cigarettes both before and after antigen administration resulted in a similar lack of effect on the PFC capabilities of the experimental animals. The dose of smoke was then increased by exposing the animals to three 1A1 cigarettes twice each day. Table 10 shows the results of experiments in which the animals were injected with KLH at time 0 and smoke was given at the same time and for the following 5 days. It can be seen that such an increase in smoke exposure did not have a significant effect on the number of PFC since the sham-smoked animals also showed a decrease in immune responsiveness compared to control, untreated mice. Table 11 shows an experiment in which mice were exposed to three 1A1 cigarettes before the administration of antigen and then rested until the time of assay, day 10. Again, it can be seen that the apparent diminution of immune responsiveness is not significant since the sham-smoked animals also showed a decreased immune response. Not shown are the results of a similar experiment in which mice were also exposed to the three 1A1 cigarettes after antigen injection. In this case as well there was no clear cut effect of smoking on the ability of the mice to respond to KLH. Table 12 presents data from an experiment in which mice were exposed to smoke from three 1A1 cigarettes and antigen was injected one day later. This experiment provides some indication (which must be interpreted cautiously) that the three 1A1 cigarettes had an immunosuppressive effect on both direct and indirect PFC. It should be pointed out that these animals were larger than any used previously, and, therefore, could have tolerated the stress effects of the smoking machine better than smaller animals. The immune response of smoked animals was suppressed by about 30% whereas sham-smoked animals showed only 10% suppression compared to the untreated controls. Several possible factors could explain the reproducible lack of effect of the 1A1 cigarettes on the immune response. First, the nature of the components of the 1A1 cigarette may preclude them being immunosuppressive, since these cigarettes are low nicotine-containing. Second, the duration of exposure to smoke may have been too short to see a systemic suppressive immunosuppressive effect. Third, cigarette smoke may not be immunosuppressive.

We next turned our attention to the effects of 1R1 cigarettes on PFC production since these cigarettes are high nicotine-containing. Table 13 shows the results of an experiment in which mice were exposed to a single 1R1 cigarette starting at the time of antigen administration. All of the conditions used for smoke exposure were the same as those described for the 1A1 cigarettes. It can be seen that there was a significant suppression of the direct plaque-forming cell number in smoked animals. Smoked animals showed a 76% suppression of PFC compared to untreated bench controls. In this experiment, as in preceeding ones, there was also about a 10-15% suppression in the sham-smoked animals. However, there is certainly a significant reduction of PFC in smoked animals compared to these controls. Table 14 shows that if the animals were exposed to smoke before antigen injection and then allowed to rest (i.e., no addition smoke, but being

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placed on the smoking machine) until the day of assay there appears to be restoration of immune responsiveness. It can be seen that there is only 40% suppression of responsiveness if the animals are not exposed to smoke for the 4-5 days after antigen administration. These results suggest that the effects of smoking may be reversible. Table 15 shows the effect of increasing smoke exposure (2 1R1 cigarettes) in immune responsiveness. In this experiment the animals were given antigen and started on the smoking protocol at the same time. The data indicate that exposure to smoke for 5 days resulted in a 98% suppression of direct PFC and complete suppression of the appearance of indirect PFC. Again I must point out that there was an effect of stress on control animals. Tables 16 and 17 show the effects of exposing the animals to smoke before antigen injection and then withholding or continuing the smoking protocol until the day of assay. Table 16 shows that there is an approximate 50% recovery of the PFC number in animals which were no longer exposed to smoke. Table 17, however, shows that continued exposure to smoke after antigen injection results in almost complete suppression of the appearance of plaque-forming cells. Table 18 shows the effect of increasing the number of 1R1 cigarettes to 3 at each exposure. In this experiment the mice were injected with KLH and started on the smoking protocol at the same time. Again there was almost complete suppression of both direct and indirect PFC in the smoked animals. Tables 19 and 20 give the results of experiments in which the animals were exposed to smoke from three 1R1 cigarettes for 5 days preceeding antigen injection and were either exposed to smoke for an additional 5 days or withdrawn from the smoking protocol. If smoke was continued (Table 20), then complete suppression of PFC appearance was obtained. On the other hand, if smoked was withheld, there appeared to be recovery of immune responsiveness (Table 19). In view of these findings, we decided that for the near future all additional experiments on the effects of cigarette smoke would employ 1R1 cigarettes.

suppression compared to the untreated controls. Several possible factors could

Preliminary experiments were carried out on the effect of smoke exposure on serum hemagglutinating antibody. Animals were exposed to three 1R1 cigarettes for varying periods up to 28 days. At intervals of 7 days, groups of 10 mice each were injected with KLH and were then bled 7 days later. Table 21 shows that there was significant diminution of serum antibody against KLH in animals even after one week of smoke exposure. Continued exposure resulted in continued suppression of antibody formation. The results in Table 22 indicate that there is recovery of the serum antibody response in animals which had been removed from the smoking protocol. These effects are similar to that observed in the PFC response to 1R1 cigarettes. One experiment was also performed on the effect of smoking on the secondary response. In this case, animals were injected on day 0 and then again on day 28 without ever having been exposed to smoke. Groups of 10 mice each were then exposed to three 1R1 cigarettes for either 4, 6 or 8 days following secondary antigen stimulation and their serum antibody levels were determined. The data in Table 23 indicate that there was no immunosuppressive effect on the in vivo secondary response. This finding may possibly be explained by the fact that the population of memory cells generated as a consequence of primary immunization is resistant to the immunosuppressive effect of cigarette smoke. Additional experiments in which animals are smoked before secondary immunization are now in progress.

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placed on the smoking machine until the day of assay when spleens were removed.

Preliminary experiments were also undertaken to determine if there was a similar suppressive effect of cigarette smoke on T-cell functions. In these experiments mice were exposed to cigarette smoke from three 1R1 cigarettes, two times each day for either 7 or 15 days consecutively. At the end of this period, the mice were sacrificed, their spleens removed, cell suspension prepared and set up in culture. Cultures were stimulated with optimal dilutions of either Concanavalin A (2.0 µg) or Phytohemagglutinin (25 µg) and incubated for 48 hrs. After this period, 1 µCi of ^{3}H-thymidine was added to mitogen-stimulated and unstimulated control cultures and incubation was continued for an additional 24 hrs. After this, cultures were harvested, the cells were washed, precipitated with TCA and prepared for liquid scintillation counting. The data in Tables 24 and 25 indicate that there was no suppression of T-cell function as a consequence of exposure to cigarette smoke. On the contrary, the data in Table 24 suggests that there is an enhancement of mitogenic activity in smoke exposed animals. Whether this enhancement is real will await reproduction of the experiment. The lack of a suppressive effect on T-cell function was not unexpected in such short term smoke exposure protocols since similar observations had been reported previously.

Finally, I should like to point out three areas of research which are now in progress and have not been included in this report. We have been exposing several groups of mice to both 1A1 and 1R1 cigarettes on a routine basis of 5 days/week, 2 exposures/day since October, 1974 when the machine first arrived. At some time in the near future, these animals will be subjected to similar assays described to determine the effects of chronic smoke exposure. Secondly, we are exposing a group of mice to the 1R1 cigarettes in the presence of a Cambridge filter to evaluate the effect of the gas phase of smoke. Lastly, we have established a collaborative effort with Dr. M. Robinowitz, a member of the Department of Pathology, who is interested in evaluating pathological changes in animals exposed to smoke. His many interests are concerned with detecting inflammatory changes and detecting pigment in macrophages found in terminal bronchioles. For these studies, we are giving him lungs from mice used in our experimental protocols.

We feel that we now have the beginnings to undertake comprehensive studies of mechanisms of defects induced by cigarette smoke. The observation that 1R1 cigarettes caused suppression of immune responsiveness in the intact animal leads us to ask where or upon which cell type the suppressive effect is operating. Once determined, we can shift our emphasis to the molecular level.

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Table 1

Effect of Acute Exposure to Cigarette Smoke on the In Vivo Primary PFC Response

Antigen Given at Time of Initiation of Smoking

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (6)	15.26	13.13	0.075	1,603	N.D.
Sham-smoked (6)	13.36	14.95	0.094	1,750	N.D.
Bench Control (4)	14.28	15.31	0.104	2,016	N.D.

N.D. = Not Determined

Conditions:

Cigarette: Kentucky Reference 1A1

Exposure: 1 Cigarette, 2 times/day, 0-5 days

Antigen: Injected on day 0

Assay: Day 5

Additional Information: Antigen given day 0, smoke begun day 0

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Source: https://www.industrydocuments.ucsf.edu/docs/gyum0000

Table 2

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Smoke Before Antigen
No Smoke After Antigen

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	14.38	14.58	0.104	2,022	677
Sham-smoked (5)	14.60	13.43	0.102	2,250	424
Bench Control (3)	14.53	14.06	0.108	2,246	512

Conditions:

Cigarette: Kentucky Reference 1A1

Exposure: 1 Cigarette, 2 times/day, 0-7 days

Antigen: Injected on day 7

Assay: Day 12

Additional Information: Animals rested (no smoke) days 7-12

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Source: https://www.industrydocuments.ucsf.edu/docs/gyam0000

Table 3

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Smoke Before Antigen
Smoke After Antigen

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	14.00	14.32	0.100	1,780	585
Sham-smoked (5)	14.60	13.43	0.102	1,921	521
Bench Control (3)	14.52	14.96	0.106	2,142	553

Conditions:

Cigarette: Kentucky Reference 1A1

Exposure: 1 Cigarette, 2 times/day, 0-7 days

Antigen: Injected on day 7

Assay: Day 12

Additional Information: Animals smoked on days 7-12

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Table 4

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Antigen Injected Day 0
Smoke Begun 1 Day Later

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (6)	15.93	14.95	0.098	1,716	N.D.
Sham-smoked (4)	15.03	15.80	0.106	1,640	N.D.
Bench Control (3)	15.61	15.85	0.112	1,987	N.D.

N.D. = Not Determined

Conditions:

Cigarette: Kentucky Reference 1A1

Exposure: 1 Cigarette, 2 times/day, days 1-5

Antigen: Injected on day 0

Assay: Day 5

Additional Information: Antigen given day 0, smoke begun 1 day later

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Table 5

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Antigen Injected Day 0
Smoke Begun 2 Days Later

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	17.10	15.84	0.113	1,653	N.D.
Sham-smoked (5)	16.90	16.47	0.126	1,735	N.D.
Bench Control (3)	17.23	17.98	0.124	2,347	N.D.

N.D. = Not Determined

Conditions:

Cigarette: Kentucky Reference 1A1

Exposure: 1 Cigarette, 2 times/day, days 2-5

Antigen: Injected day 0

Assay: Day 5

Additional Information: Antigen given day 0, smoke begun 2 days later

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Source: https://www.industrydocuments.ucsf.edu/docs/gyum0000

Table 6

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Antigen Injected Day 0
Smoke Begun 3 Days Later

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	17.58	16.34	0.116	1,986	N.D.
Sham-smoked (6)	16.32	15.76	0.122	2,078	N.D.
Bench Control (4)	17.23	16.94	0.112	2,272	N.D.

N.D. = Not Determined

Conditions:

Cigarette: Kentucky Reference 1A1

Exposure: 1 Cigarette, 2 times/day, days 3-5

Antigen: Injected day 0

Assay: Day 5

Additional Information: Antigen given day 0, smoke begun 3 days later

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Source: https://www.industrydocuments.ucsf.edu/docs/gydm0000

Table 7

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Antigen Injected Day 0
Smoke Begun 4 Days Later

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	15.48	15.86	0.112	1,516	N.D.
Sham-smoked (5)	18.16	16.66	0.117	1,677	N.D.
Bench Control (4)	16.73	17.21	0.123	2,034	N.D.

N.D. = Not Determined

Conditions:

Cigarette: Kentucky Reference 1A1

Exposure: 1 Cigarette, 2 times/day, days 4-5

Antigen: Injected on day 0

Assay: Day 5

Additional Information: Antigen given day 0, smoke begun 4 days later

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Source: https://www.industrydocuments.ucsf.edu/docs/gydf0000

Table 8

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Antigen Given 1 Day Before Smoke Begun
(2 Cigarettes)

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	14.92	12.82	0.083	2,101	521
Sham-smoked (5)	14.96	14.20	0.101	2,069	586
Bench Control (4)	14.85	15.13	0.106	2,393	647

Conditions:

Cigarette: Kentucky Reference 1A1

Exposure: 2 Cigarettes, 2 times/day, 0-5 days

Antigen: Injected on day 0

Assay: Day 5

Additional Information: Antigen given 1 day before smoke begun

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Source: https://www.industrydocuments.ucsf.edu/docs/gydm0060

Table 9

Effect of Acute Exposure to Cigarette Smoke on the In Vivo Primary PFC Response

Smoke Before Antigen (2 Cigarettes)
No Smoke After Antigen

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	15.02	12.28	0.082	1,966	406
Sham-smoked (4)	15.00	14.20	0.078	1,872	421
Bench Control (4)	15.61	16.02	0.102	2,153	648

Conditions:

Cigarette:	Kentucky Reference 1A1
Exposure:	2 Cigarettes, 2 times/day, 0-5 days
Antigen:	Injected on day 6
Antigen:	Injected on day 0
Assay:	Day 11
Additional Information:	Animals rested (no smoke) days 6-11

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Source: https://www.industrydocuments.ucsf.edu/docs/gydm0088

Table 9

Table 10

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Smoke Before Antigen (3 Cigarettes)
Antigen and Smoke Begun on Day 0
(3 Cigarettes)

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	12.44	12.24	0.075	1,575	N.D.
Sham-smoked (5)	12.86	13.40	0.091	1,627	N.D.
Bench Control (5)	12.68	13.01	0.086	1,964	N.D.

N.D. = Not Determined

Conditions:

Cigarette: Kentucky Reference 1A1

Exposure: 3 Cigarettes, 2 times/day, days 0-5

Antigen: Injected on day 0

Assay: Day 5

Additional Information: Antigen and smoke begun on day 0

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Source: https://www.industrydocuments.ucsf.edu/docs/gydm0000

Table 11

Effect of Acute Exposure to Cigarette Smoke on the In Vivo Primary PFC Response

Smoke Before Antigen (3 Cigarettes)
No Smoke After Antigen

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	12.40	12.74	0.092	1,531	N.D.
Sham-smoked (5)	12.40	13.53	0.100	1,742	N.D.
Bench Control (5)	12.81	13.92	0.106	2,165	N.D.

N.D. = Not Determined

Conditions:

Cigarette: Kentucky Reference 1A1

Exposure: 3 Cigarettes, 2 times/day, day 0-5

Antigen: Injected day 5

Assay: Day 10

Additional Information: Animals rested (no smoke) days 5-10

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Source: https://www.industrydocuments.ucsf.edu/docs/gydm0000

Table 12

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Antigen Injected 1 Day After Smoke Begun
(3 Cigarettes)

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	17.92	16.82	0.112	1,467	424
Sham-smoked (5)	18.93	17.96	0.111	1,890	591
Bench Control (3)	18.21	19.03	0.121	2,093	638

Conditions:

Cigarette: Kentucky Reference 1A1

Exposure: 3 Cigarettes, 2 times/day, days 0-5

Antigen: Injected day 1

Assay: Day 5

Additional Information: Antigen injected 1 day after smoke begun

100345816

Source: https://www.industrydocuments.ucsf.edu/docs/gyhh0000

Table 13

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Antigen and Smoke Given at Same Time
(1 Cigarette)

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	15.32	13.36	0.089	486	N.D.
Sham-smoked (5)	15.06	12.86	0.079	1,827	N.D.
Bench Control (5)	15.16	16.10	0.108	2,084	N.D.

N.D. = Not Determined

Conditions:

Cigarette: Kentucky Reference 1R1

Exposure: 1 Cigarette, 2 times/day, days 0-5

Antigen: Injected day 0

Assay: Day 5

Additional Information: None

100354817

Source: https://www.industrydocuments.ucsf.edu/docs/

Table 14

Effect of Acute Exposure to Cigarette Smoke
On the In Vivo Primary PFC Response

Smoked Before Antigen (1 Cigarette)
No Smoke After Antigen

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	15.26	14.42	0.084	1,143	N.D.
Sham-smoked (5)	15.20	12.68	0.062	1,621	N.D.
Beach Control (5)	15.24	16.38	0.101	1,857	N.D.

N.D. = Not Determined

Conditions:

Cigarette: Kentucky Reference 1R1

Exposure: 1 Cigarette, 2 times/day, days 0-3

Antigen: Injected on day 5

Assay: Day 10

Additional Information: Animals rested (no smoke) days 5-10

100354581

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

Table 15

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Antigen and Smoke Given at Same Time
(2 Cigarettes)

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	15.40	14.06	0.084	42	0
Sham-smoked (5)	15.40	13.80	0.108	1,415	486
Bench Control (5)	15.42	16.82	0.115	1,986	621

Conditions:

Cigarette: Kentucky Reference 1R1

Exposure: 2 Cigarettes, 2 times/day, days 0-5

Antigen: Injected day 0

Assay: Day 5

Additional Information: Antigen and smoke given same time

100345819

Source: https://www.industrydocuments.ucsf.edu/docs/gydm0000

Table 16

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Animals Smoked Before Antigen (2 Cigarettes)
Animals Rested (No Smoke) After Antigen

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (6)	15.34	14.16	0.113	1,063	286
Sham-smoked (4)	15.25	13.65	0.110	1,996	451
Bench Control (4)	15.28	16.82	0.121	2,154	683

Conditions:

Cigarette: Kentucky Reference 1R1

Exposure: 2 Cigarettes, 2 times/day, days 0-5

Antigen: Injected on day 5

Assay: Day 10

Additional Information: Animals rested (no smoke) days 5-10

100345820

Source: https://www.industrydocuments.ucsf.edu/docs/gydm0000

Table 17

Effect of Acute Exposure to Cigarette Smoke on the In Vivo Primary PFC Response

Animals Smoked Before Antigen (2 Cigarettes)
Animals Smoked After Antigen (2 Cigarettes)

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (6)	12.62	11.43	0.071	1.55	0
Sham-smoked (8)	12.72	11.90	0.088	1,743	387
Bench Control (3)	12.68	13.72	0.124	2,087	592

Conditions:

Cigarette: Kentucky Reference 1R1

Exposure: 2 Cigarettes, 2 times/day, days 0-5

Antigen: Injected on day 5

Assay: Day 10

Additional Information: Smoke continued until day 10

100345821

Source: https://www.industrydocuments.ucsf.edu/docs/gydm0000

Table 18

Effect of Acute Exposure to Cigarette Smoke on the In Vivo Primary PFC Response

Animals Smoked Before Antigen (2 Cigarettes)
Antigen and Smoke Given at Day 0
(3 Cigarettes)

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (6)	16.06	15.22	0.126	21	0
Sham-smoked (4)	16.20	15.76	0.153	2,671	821
Bench Control (5)	16.23	17.20	0.162	2,866	924

Conditions:

Cigarette: Kentucky Reference 1R1

Exposure: 3 Cigarettes, 2 times/day, days 0-5

Antigen: Injected day 0

Assay: Day 5

Additional Information: None

100345822

Source: https://www.industrydocuments.ucsf.edu/docs/xydm0000

Table 19

Effect of Acute Exposure to Cigarette Smoke on the In Vivo Primary PFC Response

Smoke Before Antigen (3 Cigarettes)
No Smoke After Antigen

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (6)	14.28	13.81	0.082	1,468	N.D.
Sham-smoked (4)	14.31	13.90	0.084	2,320	N.D.
Bench Control (4)	14.24	15.16	0.099	2,613	N.D.

N.D. = Not Determined

Conditions:

Cigarette: Kentucky Reference 1R1

Exposure: 3 Cigarettes, 2 times/day, days 0-5

Antigen: Injected on day 5

Assay: Day 10

Additional Information: Animals rested (no smoke) days 5-10

10035823

Source: https://www.industrydocuments.ucsf.edu/docs/fqdm0000

Table 20

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary PFC Response

Smoked Before Antigen (3 Cigarettes)
Smoked After Antigen (3 Cigarettes)

Group (n)	Body Weight at Start	Body Weight at Assay	Spleen Weight at Assay	Direct PFC/10^6 cells	Indirect PFC/10^6 cells
Smoked (5)	15.16	14.21	0.099	26	N.D.
Sham-smoked (5)	15.14	14.38	0.110	2,216	N.D.
Bench Control (5)	15.32	16.07	0.123	2,408	N.D.

N.D. = Not Determined

Conditions:

Cigarette:	Kentucky Reference 1R1
Exposure:	3 Cigarettes, 2 times/day, days 0-5
Antigen:	Injected on day 5
Assay:	Day 10
Additional Information:	Animals smoked days 5-10

100345824

Source: https://www.industrydocuments.ucsf.edu/docs/gydm0000

Table 21

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Primary Serum Antibody Response

Smoked Before Antigen (3 Cigarettes)
Smoked After Antigen (3 Cigarettes)

Day Antigen Injected	Day of Assay	HA Titer (Log_2)		
		Smoked	Sham-smoked	Bench Control
0	7	4	8	9
7	14	4	9	10
14	21	4	8	9
21	28	3	8	9
28	35	3	9	10

Conditions:
10 mice/group
Cigarette: Kentucky Reference 1R1
Antigen: Injected on day 0
Exposure: 3 Cigarettes, 2 times/day, day 0-35

Additional Information: Animals exposed to smoke throughout entire period.

100345825

Source: https://www.industrydocuments.ucsf.edu/docs/gydm0000

Table 22

Effect of Acute Exposure to Cigarette Smoke on the In Vivo Primary Serum Antibody Response

Day Antigen Injected	Day of Assay	Smoked	HA Titer (Log_2) Sham-smoked	Bench Control
0	7	4	8	9
7	14	6	9	10
14	21	8	10	10
21	28	9	9	10
28	35	9	9	10

Conditions: 10 mice/group

Cigarette: Kentucky Reference 1R1

Exposure: 3 Cigarettes, 2 times/day, days 0-7

Additional Information: Animals not smoked from days 7-35

1003545826

Source: https://www.industrydocuments.ucsf.edu/docs/gydm0000

Table 23

Effect of Acute Exposure to Cigarette Smoke
on the In Vivo Secondary Serum Antibody Response

Days of Smoke Exposure	HA Titer (Log_2)		
	Smoked	Sham-smoked	Bench Control
28-35	11	11	12
30-35	11	11	12
32-35	11	11	12

Conditions: 10 mice/group
All mice injected day 0
All mice reinjected day 28
Assay done on day 35

Cigarette: Kentucky Reference 1R1

Exposure: 3 Cigarettes, 2 times/day

1003545827

Source: https://www.industrydocuments.ucsf.edu/docs/gydm0000

Table 24

Effect of Acute Exposure (7 Days) to Smoke on the Response to Mitogenic Stimulation

Group	^{3}H-Thymidine Incorporated (cpm) Concanavalin A		Phytohemagglutinin	
Unstimulated	1,843		1,843	
Smoked	35,520	(19.3)	23,626	(12.8)
Unstimulated	1,935		1,935	
Sham-smoked	35,873	(18.5)	19,021	(9.8)
Unstimulated	2,321		2,321	
Bench Control	29,304	(12.62)	24,270	(10.5)

Conditions: 16 mice in smoked group
8 mice in sham-smoked and bench control

Cigarette: Kentucky Reference 1R1

Exposure: 3 Cigarettes, 2 times/day, 7 days

Additional Information: Spleens removed on day 7 and placed in culture with mitogen (25µg PEA, 2.0µg ConA) for 72 hrs. Pulsed for final 24 hrs. with 1.0µCi ^{3}H-thymidine.

100354582

Source: https://www.industrydocuments.ucsf.edu/docs/gydm0000

Table 25

Effect of Acute Exposure (15 Days) to Smoke on the Response to Mitogenic Stimulation

Group	^{3}H-Thymidine Incorporated (cpm)			
	Concanavalin A		Phytohemagglutinin	
Unstimulated	4,838	(11.4)	4,838	(4.8)
Smoked	55,319		23,363	
Unstimulated	4,612	(13.6)	4,612	(4.8)
Sham-smoked	62,660		22,074	
Unstimulated	4,852	(13.0)	4,852	(4.4)
Bench Control	63,266		21,316	

Conditions: 16 mice in smoked group
8 mice in sham-smoked and bench control

Cigarette: Kentucky Reference 1R1

Exposure: 3 Cigarettes, 2 times/day, 15 days

Additional Information: Assay as indicated in Table 24.

1003545829

Source: https://www.industrydocuments.ucsf.edu/docs/gydm0000